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D E C L A R A T I O N

In the matter of U. S. Patent
Application Ser. No. 09/601,658
in the name of Osamu SAKANAKA
et al.

I, Kumi HIRANO, of Kyowa Patent and Law Office, 2-3, Marunouchi 3-Chome, Chiyoda-Ku, Tokyo-To, Japan, declare and say:

that I am thoroughly conversant with both the Japanese and English languages; and

that the attached document represents a true English translation of the certified copy of Japanese Patent Application No. 1998/26257 filed on February 6, 1998 originally filed as a priority document with respect to the above captioned U. S. Patent Application.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: August 21, 2001

Kumi Hirano

Kumi HIRANO

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Title of the Invention: NOVEL ANTIFUNGAL COMPOUND AND METHOD FOR PRODUCING THE SAME

Number of Claim(s): 9

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Specification	1
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(Translation)

SPECIFICATION 10-26257

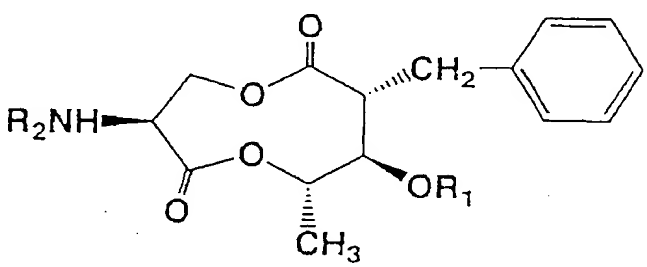
1. TITLE OF THE INVENTION

NOVEL ANTIFUNGAL COMPOUND AND METHOD FOR PRODUCING THE SAME

2. CLAIMS:

1. A compound represented by formula (1) or a salt thereof:

[Formula 1]



(1)

wherein

R_1 represents isobutyryl, tigloyl, isovaleryl, or 2-methylbutyryl;

R_2 represents a hydrogen atom, an aromatic carboxylic acid residue (a 3-hydroxypicolinic acid residue and a 3-hydroxy-4-methoxypicolinic acid residue are excluded) or a protective group of amino.

2. The compound or salt thereof according to claim 1, wherein R_2 is any one of a benzoic acid residue having a substituent, a picolinic acid residue having a substituent (a 3-hydroxypicolinic acid residue and a 3-hydroxy-4- methoxypicolinic acid residue are excluded), a nicotinic acid residue having a substituent, 4-quinolinecarboxylic acid residue having a substituent, 5-pyrimidine carboxylic acid residue having a substituent, and a 2-quinoxalinecarboxylic acid residue having a substituent.

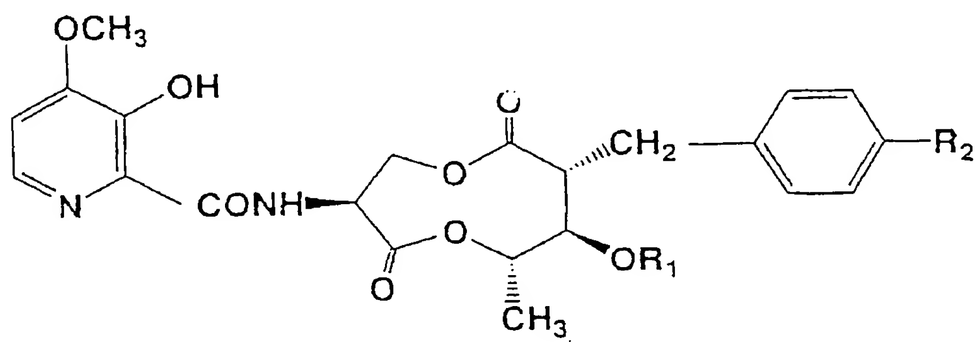
3. The compound or salt thereof according to claim 1, wherein R_2 is any one of a 2-hydroxybenzoic acid residue having a substituent, a 3-hydroxypicolinic acid residue having a substituent (a 3-hydroxypicolinic acid residue and a 3-hydroxy-4-methoxypicolinic acid residue are excluded), a 2-hydroxynicotinic acid residue having a substituent, a 3-hydroxy-4-quinolinecarboxylic acid residue having a substituent, a 4-hydroxy-5-pyrimidinecarboxylic acid residue having a substituent, and a 3-hydroxy-2-quinoxalinecarboxylic acid residue having a substituent.

4. The compound or salt thereof according to claim 1, wherein R_2 is 3-acyloxy-4-methoxypicolinyl.

5. The compound or salt thereof according to claim 1, wherein R_2 is 3-acetoxy-4-methoxypicolinyl.

6. A compound represented by formula (2) or a salt thereof:

[Formula 2]



(2)

wherein

R_1 represents isobutyryl, tigloyl, isovaleryl, or 2-methylbutyryl;

R_2 represents nitro, amino, acylamino, or N,N-dialkylamino.

7. The compound or salt thereof according to claim 6, wherein R_2 is formylamino or N,N-dimethylamino.

8. An antifungal agent comprising the compound or salt thereof according to any one of claims 1 to 7 as the active component.

9. A method for producing the compound according to claim 1, wherein R_2 is a hydrogen atom, said method comprising the steps of:

chlorinating a fermented product UK-2 with a chlorinating agent; and

etherifying the chlorination product with a lower alcohol.

[Detailed Description of the Invention]

[0001]

[Technical Field]

The present invention relates to a novel compound having antifungal activity, a method for producing the same, and use thereof.

[0002]

[Prior Art]

Yeasts and filamentous fungi, which are called fungi, are eukaryotes while bacteria are prokaryotes. Some fungi among these ones are pathogenic to human beings and non-human animals and have been regarded as being responsible for fungal infectious diseases. The pathogenicity of fungi is on the whole weak. However, fungi often bring about grave condition in patients having lowered resistance thereto. This has led to an expectation of the development of novel pharmaceuticals useful for the treatment of these diseases.

[0003]

Some fungi are known as being pathogenic, and the novel development of novel antifungal agents for agricultural and gardening applications has been required associated with the control of plant diseases. Further, in reflection of recent housing circumstances, the invasion of filamentous fungi into housing brings about such

conditions as an allergy to human beings. The development of novel antifungal agents for preventing the occurrence of such symptoms has been desired in the art.

[0004]

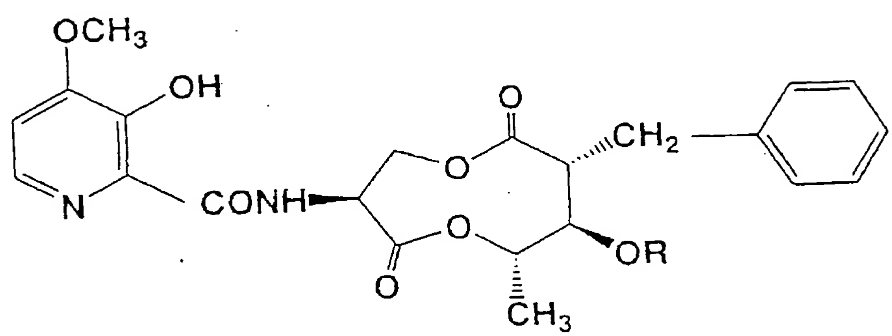
With a view to overcoming these problems, various antifungal agents have been developed with certain success. However, the development of antifungal agents, which are highly effective and safe, has been particularly desired.

[0005]

On the other hand, a culture solution and a culture of Streptoverticillium sp. SAM 2084 strain, under FERM P-14154 with National Institute of Bioscience and Human Technology, Agency of Industrial Science & Technology, which belongs to streptoverticillium, produce antifungal antibiotics. Compounds represented by formula (3) are generally referred to as UK-2.

[0006]

[Formula 3]



[0007]

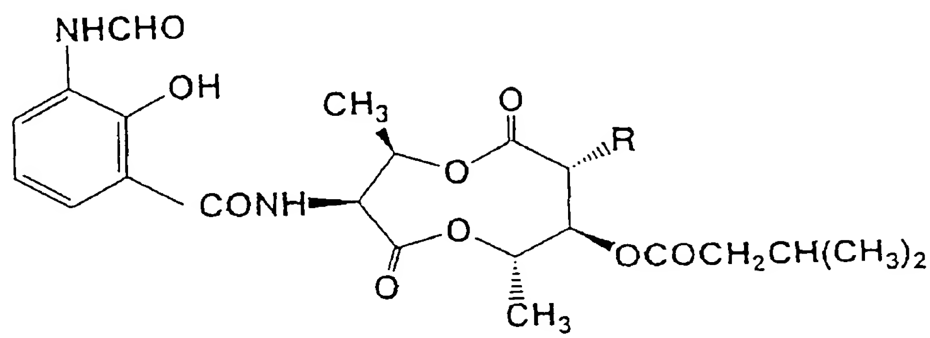
wherein:

R represents a straight-chain or branched saturated aliphatic hydrocarbon group or unsaturated aliphatic hydrocarbon group. For example, as compared with antimycins which have a dilactone structure with a nine-membered ring, UK-2 has the same or higher antimicrobial activity against fungi including filamentous fungi, such as

Aspergillus, Penicillium, Mucor, Cladosporium, Rhizopus, Sclerotina, and Tricoderma, and has much lower cytotoxicity against culture cells, such as P388. Therefore, UK-2 has led to an expectation for usefulness thereof.

[0008]

[Formula 4]



Antimycin A $R= -(CH_2)_5CH_3$

Antimycin A_3 $R= -(CH_2)_3CH_3$

[0009]

The above-described UK-2 consists of an affinous group which includes UK-2A, UK-2B, UK-2C, and UK-2D. It has been known that each of them can be separated and purified lithomatographically, according to Japanese Patent Laid-Open No. 233165/1995, The Journal of Antibiotics No. 49, 639/1996 and 49, 1226/1996.

It has been apparent that, according to subsequent researches, UK-2 has very strong activity against rice blight bacteria. Therefore, it has led to an expectation for its usefulness as antifungal agents for agricultural and gardening applications.

[0010]

However, anti-rice blight agents comprising UK-2 have not performed any effects in the field, although they show considerable effects in a pot test in a greenhouse. Therefore, there has been desired the improvement of such agents.

[0011]

[Problems to be Solved by The Invention]

The best solution by the invention is to obtain materials that have high antifungal activity and safety. Various diseases caused by fungi seriously damage the health of human beings and non-human animals and agriculture. Therefore, it has always been desired to find compounds being effective against fungi, antifungal agents comprising the compounds as the active component, and an effective method for producing the same.

[0012]

Furthermore, the present inventors established a presumption that UK-2, a fermented product, lacks stability to light and, therefore, it cannot perform in the field any effects that it has shown in a greenhouse despite its strong antifungal activity as mentioned above.

The present inventors paid attention to the above-mentioned points and began their researches on the creation of novel derivatives whose starting materials were UK-2 compounds by using chemical synthetic methods.

[0013]

[Methods to Solve the Problems]

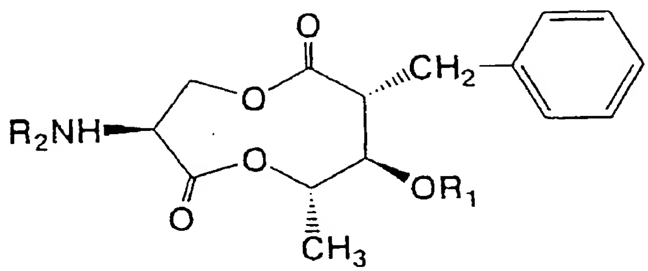
As mentioned above, the present inventors conducted researches on novel derivatives having stronger antifungal activity by using UK-2, a fermented product, as a lead compound and by using chemical synthetic methods. As a result, the present inventors succeeded in finding some novel derivatives having novel excellent antifungal activity and in providing effective methods for the synthesis of the derivatives, thereby accomplishing the invention. Furthermore, it is an object of the present invention to provide the use of the derivatives as novel antifungal agents comprising such compounds as UK-2 as the active component.

[0014]

The compound or salt thereof according to the present invention, represented by formula (1), is provided:

[0015]

[Formula 5]



(1)

[0016]

wherein

R_1 represents isobutyryl, tigloyl, isovaleryl, or 2-methylbutyryl; and

R_2 represents a hydrogen atom, an aromatic carboxylic acid residue (a 3-hydroxypicolinic acid residue and a 3-hydroxy-4-methoxypicolinic acid residue are excluded), or a protective group of amino.

[0017]

In the above formula (1), the aromatic carboxylic acid residue is preferably a benzoic acid residue having a substituent, a picolinic acid residue having a substituent (a 3-hydroxypicolinic acid residue and a 3-hydroxy-4-methoxypicolinic acid residue are excluded), a nicotinic acid residue having a substituent, a 4-quinolinecarboxylic acid residue having a substituent, a 5-pyrimidine carboxylic acid residue having a substituent, and a 2-quinoxalinecarboxylic acid residue having substituent, and, more preferably, a 2-hydroxybenzoic acid residue having a substituent, a 3-hydroxypicolinic acid residue having a substituent (a 3-hydroxy-4-methoxypicolinic acid residue is excluded), a 2-hydroxynicotinic acid residue having a substituent, a 3-hydroxy-4-quinolinecarboxylic acid residue having a substituent, a 4-hydroxy-5-pyrimidinecarboxylic acid residue having a substituent, a 3-hydroxy-2-quinoxalinecarboxylic acid residue having a substituent, a 3-acyloxy-4-methoxypicolinic acid residue, and a 3-acetoxy-4-methoxypicolinic acid residue.

[0018]

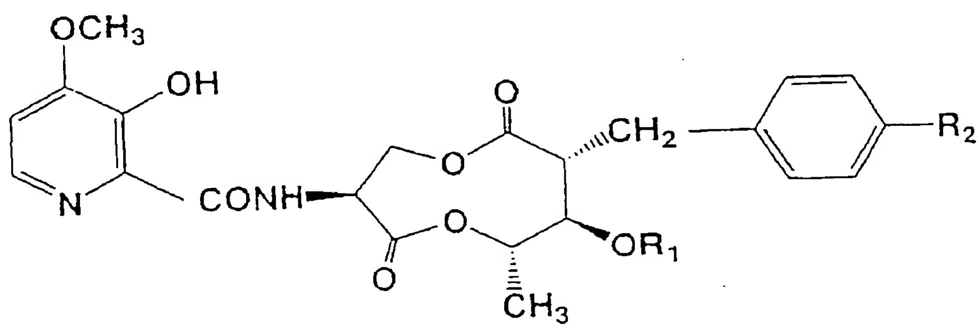
Likewise, a protective group of amino according to the above formula (1) needs to be a protective group, among ordinary ones of amino, which can be separated and removed depending on the reduction conditions or by oxidation. However, it is preferably benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, methoxycarbonyl, and t-butyloxycarbonyl, and, more preferably, benzyloxycarbonyl.

[0019]

The compound or salt thereof according to the present invention, represented by formula (2), is provided:

[0020]

[Formula 6]



(2)

[0021]

wherein

R_1 represents isobutyryl, tigloyl, isovaleryl, or 2-methylbutyryl;

R_2 represents nitro, amino, acylamino, or N,N-dialkylamino.

[0022]

In the above formula (2), an acyl group of acylamino is an aliphatic carboxylic acid residue such as formyl, acetyl, or propionyl, or an aromatic carboxylic acid residue such as benzoyl, p-methoxybenzoyl, or p-nitrobenzoyl, and an alkyl group of N,N-dialkylamino is methyl or ethyl. Acylamino is preferably formyl, and

N,N-dialkylamino is preferably N,N-dimethylamino.

[0023]

The compounds or salts thereof according to the present invention, represented by formulae (1) and (2), have antifungal activity and can provide antifungal agents comprising the compounds or salts thereof as the active component.

[0024]

[Forms To Implement The Invention]

The present inventors have tried to produce more effective novel derivatives prepared from above-described effective UK-2 as a starting compound. The present invention has been made based on such trials.

[0025]

(1) A method for chemically cleaving the carboxylic acid amido bond of UK-2

In UK-2, a nine-membered lactone ring is attached to a substituted pyridine ring moiety through a carboxylic acid amido bond. The present inventors have succeeded in obtaining a nine-membered ring lactone having an amino group by chemically cleaving the carboxylic acid amido bond. This amino compound may be used as an important intermediate for the production of UK-2 derivatives. The present inventors have further succeeded in producing novel compounds useful as antifungal agents by condensing the above amino compound with an aromatic carboxylic acid different from UK-2.

[0026]

The carboxylic acid amido bond may be generally chemically cleaved by hydrolysis with an acid or an alkali. This method, however, requires treatment with a highly concentrated acid or alkali at a high temperature for a long period of time, and hence can be applied to only compounds wherein portions other than the reaction site are stable against acids or alkalis. UK-2 has three carboxylic ester bonds including the nine-membered lactone ring structure, and these bonds are easily cleaved under such hydrolysis conditions.

[0027]

Trimethyloxonium tetrafluoroborate $(CH_3)_3OBF_4$ is frequently used as a

chemical reagent for cleaving the carboxylic acid amido bond, in the compound having a very sensitive functional group, without damage to the other portions (Tetrahedron Letters, 1549, (1967)). At first, the present inventors also have applied this method to UK-2. However, the reaction did not substantially proceed, and only UK-2 of the starting compound was obtained containing a very small amount of decomposition products.

[0028]

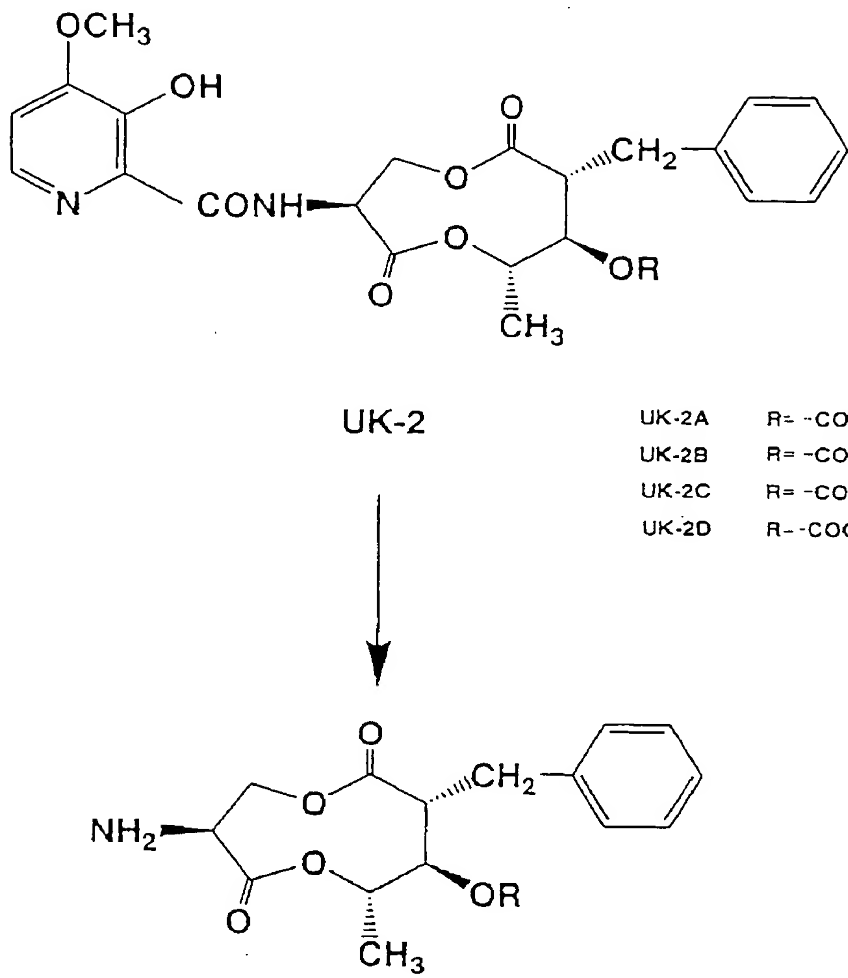
On the other hand, iminoetherification through iminochloride is known as a method for cleaving the carboxylic acid amido bond at the 6- and 7-positions respectively in penicillins and cephalosporins having a β-lactam ring which is highly susceptible to hydrolysis with acids and alkalis. Specifically, at the outset, treatment with a chlorinating agent, such as phosphorus pentachloride, is carried out to give a corresponding iminochloride. The iminochloride is treated with a lower alcohol, such as methanol, to produce an imino ether which is finally treated with water to cleave the acyl group, thereby obtaining a free amino compound at a high yield.

[0029]

The present inventors have applied this iminoetherification method to UK-2 and have succeeded in obtaining the desired amino compound (An amino compound obtained from UK-2 is generally described as UK-2-28L. When obtained from UK-2A, it is specified as UK-2A-28L). This production of the amino compound from UK-2 is first success in compounds having a chemically very unstable nine-membered dilactone ring structure, such as UK-2 and antimycins.

[0030]

[Formula 7]



UK-2

UK-2A R= $-COCH(CH_3)_2$
UK-2B R= $-COC(CH_3)=CHCH_3$
UK-2C R= $-COCH_2CH(CH_3)_2$
UK-2D R- $-COCH(CH_3)CH_2CH_3$

UK-2-28L

UK-2A-28L R= $-COCH(CH_3)_2$
UK-2B-28L R= $-COC(CH_3)=CHCH_3$
UK-2C-28L R= $-COCH_2CH(CH_3)_2$
UK-2D-28L R= $-COCH(CH_3)CH_2CH_3$

[0031]

More specific explanation about this reaction of cleaving the carboxylic acid amido is given as follows. UK-2 is dissolved in an inert organic solvent, a chlorinating agent is added to the solution, and the mixture is heated under reflux to perform a

reaction. The amount of the chlorinating agent added is 1 to 10 molar equivalents, preferably 2 to 3 molar equivalents. The reaction time is 1 to 5 hr, preferably 1 to 3 hr. The reaction temperature is 0 to 80℃, preferably 30 to 40℃. This reaction gives a corresponding iminochloro compound. After the completion of the reaction, the reaction solution is cooled to -30 to -20℃. To the cooled reaction solution is added a lower alcohol (cooled to 0 to 5℃) of weight of 10 to 100 times that of UK-2 as the starting compound, followed by a reaction. The reaction time is 1 to 15 hr, preferably 2 to 3 hr. The reaction temperature is 0 to 50℃, preferably 15 to 25℃. This gives a corresponding iminoether compound. The iminoether compound easily undergoes hydrolysis by treatment with water to produce a desired amino compound. A representative example of the chlorinating agent used is phosphorus pentachloride. Lower alcohols usable herein include methanol, ethanol, n-propyl alcohol, isopropyl alcohol, n-butyl alcohol, and isobutyl alcohol. Reagents, however, are not limited to these

[0032]

A free amino group and a dilactone structure are copresent in the UK-2-28L amino compound having nine-membered dilactone ring thus obtained. Therefore, isolation and storage for a long period of time in this form pose a problem. For this reason, preferably, the desired UK-2-28L amino compound in its free amino group is converted to a salt, for example, p-toluenesulfonate or hydrochloride, or is protected by a protective group which can be easily introduced and removed, for example, benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, methoxycarbonyl, or t-butyloxycarbonyl. The treated product obtained is purified and isolated, and, in this state, is stored. In this case, preferably, the salt or the protected amino group is returned to the free amino group immediately before use or within the reaction system, and is then used in the condensation.

[0033]

(2) A method for synthesizing an antifungal compound by acylation of UK-2-28L.

The amino compound of UK-2-28L obtained by the above process is easily reacted with any carboxylic acid, carboxylic acid chloride, carboxylic anhydride, active ester of carboxylic acid or the like. This reaction can give a corresponding carboxylic

acid amido compound. For example, the amino compound of UK-2-28L and a carboxylic acid may be treated with a dehydration condensation reagent in an inert solvent, thereby producing a carboxylic acid amido compound. Most generally well-known dehydration condensation reagents can be used for the synthesis of a compound of the present invention. Representative examples of dehydration condensation reagents usable herein are dicyclohexylcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, and a combination of dicyclohexylcarbodiimide with 1-hydroxybenzotriazole. When a carboxylic acid, whose reactivity has been activated in advance, is used, it is possible to use a method wherein the carboxylic acid is treated with thionyl chloride, phosphorus pentachloride or the like to give an acid chloride, a method wherein the carboxylic acid is reacted with a chlorocarbonic ester, phosphorus oxychloride or the like to give an acid anhydride, or a method wherein the carboxylic acid is condensed with N-hydroxysuccinimide or 2-mercaptobenzothiazole to give an active ester. These methods can also be applied to the synthesis of a compound of the present invention. The contemplated carboxylic acid amido may be easily produced by reacting the activated carboxylic acid with the compound UK-2-28L in an inert solvent under neutral or weakly basic conditions.

[0034]

These carboxylic acid amides have been demonstrated to have high antifungal activity, no phytotoxicity against various plant diseases, excellent prophylactic or therapeutic effect. Heterocyclic carboxylic acid derivatives with a hydroxyl group being present in a carbon atom adjacent to a carbon atom attached to the amido group and, in addition, having at least one nitrogen atom as the ring-constituting atom, salicylic acid derivatives which have been unsubstituted or substituted at 3- or 5-position by a nitrogen-containing group (such as nitro, formylamino, or N,N-dimethylamino), or chloro had particularly high activity.

[0035]

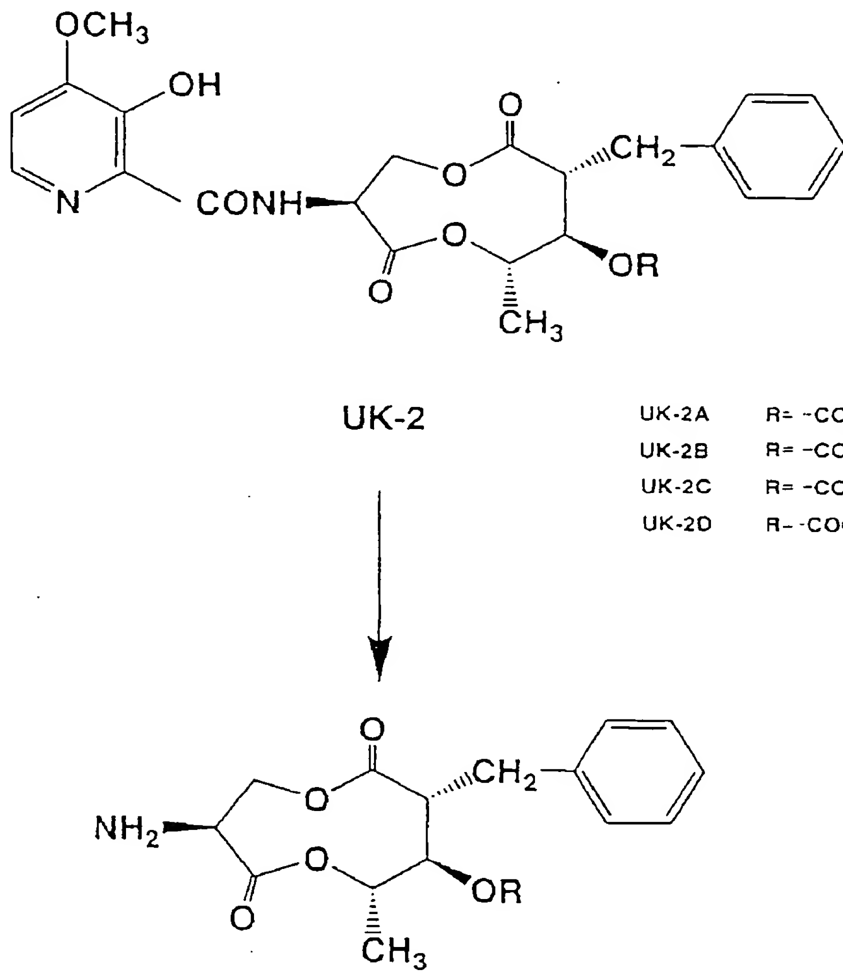
<u>(3) A method for synthesizing an antifungal compound by acylation of a 3'-position hydroxyl group of UK-2</u>

A fermented product UK-2 had a problem with being used as an agricultural chemical due to its lacking photostability, although it had excellent antifungal activity.

The present inventors have found that photostability can be substantially improved by protecting a 3'-position hydroxyl group with an acyl group.

[0036]

[Formula 8]



UK-2

UK-2A	R= $-COCH(CH_3)_2$
UK-2B	R= $-COC(CH_3)=CHCH_3$
UK-2C	R= $-COCH_2CH(CH_3)_2$
UK-2D	R- $-COCH(CH_3)CH_2CH_3$

UK-2-28L

UK-2A-28L	R= $-COCH(CH_3)_2$
UK-2B-28L	R= $-COC(CH_3)=CHCH_3$
UK-2C-28L	R= $-COCH_2CH(CH_3)_2$
UK-2D-28L	R=$-COCH(CH_3)CH_2CH_3$

[0037]

An acyl compound represented by the above formula is obtained at substantially quantitatively yields by using acylation of hydroxyl groups which are generally widely used to UK-2. Most methods for acylation of hydroxyl groups may be applied to acylation used in the present invention. For example, a combination of an carboxylic acid anhydride (for example, acetic anhydride, propionic anhydride, or an acid anhydride of benzoic acid) with a tertiary organic base, such as pyridine or triethylamine, a combination of a corresponding acid chloride (for example, acetyl chloride, propionyl chloride, pivaloyl chloride, benzoyl chloride, or succinic acid chloride) with the tertiary organic base, or a combination of a corresponding free carboxylic acid with a dehydration condensation agent, such as dicyclohexylcarbodiimide is useful in the absence or presence of an inert solvent, such as methylene chloride, chloroform, 1,4-dioxane, or tetrahydrofuran.

[0038]

The acyl compounds obtained by the above reaction according to the present invention have high antifungal activity and, at the same time, the photostability has been improved by virtue of acylation. Thus, they have properties which are favorable for use as agricultural chemicals compared with UK-2.

[0039]

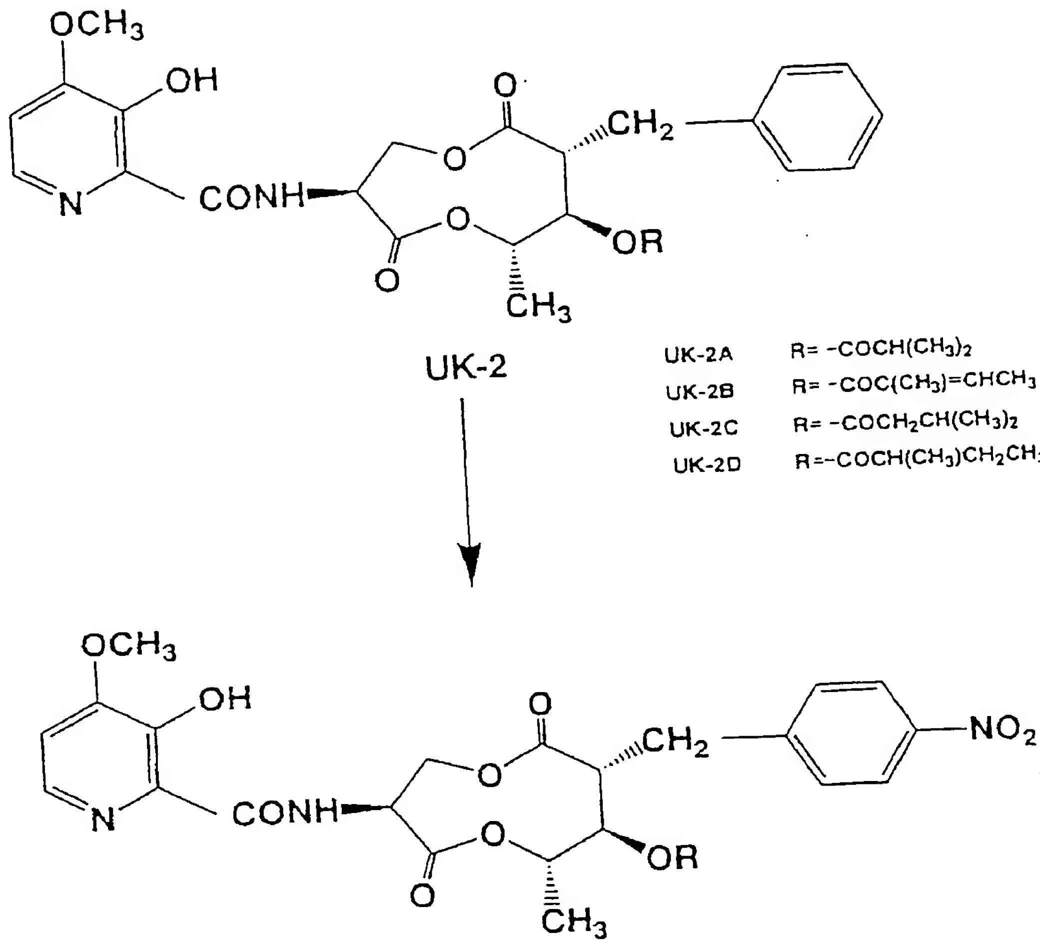
<u>(4) A method for synthesizing an antifungal compound by chemical modification of benzene ring in a benzyl group at the 2-position of UK-2.</u>

It is one of substantial chemical structural characteristics as well as a difference from antimycins that UK-2 has a benzyl group at a nine-membered ring lactone at the 2-position.

The present inventors tried to subject this benzene ring in a benzyl group to electrophilic nitro substitution on the aromatic ring and then, succeeded in producing a compound wherein a nitro group were selectively introduced into para position of the desired benzene ring in a high yield. It was found that the nitration was preferably carried out using fuming nitric acid as strong nitric agent in cooked (- 20℃ to - 50℃) methylene chloride or chloroform and that the nitration time was preferably one to two hr.

[0040]

[Formula 9]



UK-2

UK-2A	R= $-COCH(CH_3)_2$
UK-2B	R= $-COC(CH_3)=CHCH_3$
UK-2C	R= $-COCH_2CH(CH_3)_2$
UK-2D	R=$-COCH(CH_3)CH_2CH_3$

UK-2-401

UK-2A-401	R= $-COCH(CH_3)_2$
UK-2B-401	R= $-COC(CH_3)=CHCH_3$
UK-2C-401	R= $-COCH_2CH(CH_3)_2$
UK-2D-401	R=$-COCH(CH_3)CH_2CH_3$

[0041]

A chemical conversion method commonly used for normal aromatic nitro compounds may be applied to nitro compounds thus obtained. The compounds may be

subjected to a reduction to animo compounds, or more specifically, N-acylation (such as formylation or acetylation) or N-alkylation (such as N,N-dimethylation or N,N-diethylation).

[0042]

The compounds obtained by the chemical conversion according to the present invention have been found to have potent antifungal activity and excellent prophylactic or therapeutic effect against various plant diseases as a result of various tests. Therefore, the compounds are useful as active ingredients of antifungal agents for the treatment of fungal infectious diseases derived from fungi sensitive to the compounds of the present invention and, in addition, as active ingredients of antifungal agents for agricultural and gardening applications or antifungal agents for industrial applications.

[0043]

Antifungal agents, for the treatment of fungal infectious diseases, comprising the compounds according to the present invention may be prepared by combining the compounds with known medical carriers. For example, they are formed into preparations for total administration; for parenteral administration, such as hypodermic injections, intravenous injections, intramuscular injections, or suppositories, or preparations for oral administration, such as tablets, capsules, powders, or granules; or preparations for local administrarion, such as ointments, lotions or pessaries.

[0044]

Antifungal agents for agricultural and gardening applications comprising the compound according to the present invention may be prepared by using known carriers and, if necessary, incorporating proper additives. For example, they are preferably formed into solid preparations, such as powders and granules, and liquid preparations, such as solutions, emulsions, suspensions, and aerosols. The antifungal agent for agricultural and gardening applications may be used for the prevention of diseases derived from plant bacteria sensitive to the compounds of the present invention.

[0045]

The compound according to the present invention, when intended to be used as antifungal agents for industrial applications, may be prepared by using known carriers and, if necessary, incorporating proper additives. These antifungal agents for industrial

applications prevent the propagation of harmful fungi which pose a problem in general industrial products and in the course of the production of these products to prevent contamination with harmful fungi. Examples of antifungal agents for industrial applications include fungicides for the prevention of surface contamination of wood, countermeasuring agents for rotting fungi in wood products, preservatives/fungicides to be added to paints, wall coverings, and fungicides to be added in polymer processing, and fungicides to be used in processing of leather, fibers, and textiles.

[0046]

[Examples]

The present invention is described by the use of examples of practices and tests, but it is not limited to the scope of the examples.

[0047]

Example 1 (2R, 3R, 4S, 7S)-7-Amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione; and p-toluenesulfonate thereof (Compounds UK-2A-28L and UK-2A-29L represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents a hydrogen atom):

UK-2A (500 mg) was dissolved in 50 mL of methylene chloride. Pyridine (0.15 mL) and 395 mg of phosphorus pentachloride were added to the solution under ice cooling. The mixture was heated under neflux for 1.5 hr. The reaction solution was cooled to -30℃. Thereafter, 50 mL of methanol, which had been previously cooled to 0℃, was added to the reaction solution, and a reaction was allowed to proceed for 15 hr. Methylene chloride (200 mL) and 150 mL of saturated aqueous sodium hydrogencarbonate, which had been previously cooled to 0℃, were added thereto, followed by separation. The aqueous layer was extracted twice with 20 mL of dichloromethane. The combined organic layers were dried over magnesium sulfate, and concentrated under the reduced pressure. The residue was dissolved in 50 mL of ethyl acetate. A solution of 180 mg of p-toluenesulfonic acid monohydrate in ethyl acetate (50 mL) was added to the solution at room temperature. The precipitated compound UK-2A-29L was collected by filtration. The amount of the product thus obtained was 232 mg (yield 45%).

[0048]

This UK-2A-29L (87 mg) was dissolved in a mixed solution composed of methylene chloride and 5% aqueous sodium hydrogencarbonate, followed by separation. The organic layer was dried over sodium sulfate, and concentrated under the reduced pressure to obtain 51 mg (yield 86%) of the title compound UK-2A-28L.

[0049]

Compound UK-2A-29L:

^{1}H-NMR ($(CD_3)_2$ SO): δ=1.17 (6H, d, J=7.0, CH ($\underline{CH_3}$)$_2$), 1.32 (3H, d, J=5.86, 4-CH_3), 2.30 (3H, s, $\underline{CH_3}C_6H_4SO_3H$), 2.60~2.80 (3H, m, J=7.0, $\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 3.00~3.20 (1H, m, H-2), 350 (1H, bs, H-8), 4.52 (1H, dd, J=5.5, 8.4 H-8), 4.90~5.20 (3H, m, H-3, 4, 7), 7.11 (2H, d, J=7.6, $CH_3\underline{C_6H_4}SO_3H$), 7.14~7.30 (5H, m, C_6H_5), 7.48 (2H, d, J=8.1, $CH_3\underline{C_6H_4}SO_3H$)

[0050]

Compound UK-2A-28L:

^{1}H-NMR (CD_3 OD): δ=1.22 (6H, d, J=7.0, CH ($\underline{CH_3}$)$_2$), 1.32 (3H, d, J=6.1, 4-CH_3), 2.60 (1H, septet, J=7.0, $\underline{CH}$ $(CH_3)_2$), 2.76 (1H, dd, J=13.4, 4.3, $\underline{CH_2}C_6H_5$), 2.81 (1H, dd, J=13.4, 9.5, $\underline{CH_2}C_6H_5$), 3.02 (1H, td, J=4.3, 9.5, H-2), 3.82 (1H, bs, H-8), 4.41 (1H, bs, NH_2), 4.51 (1H, bs, NH_2), 4.70~5.30 (4H, m H-3, 4, 7, 8), 7.11~7.23 (5H, m, C_6H_5)

MS (EI): m/z= 363(M)

[0051]

Example 2 (2R, 3R, 4S, 7S)-7-Benzyloxycarbonylamino-2-benzyl-5,9-dioxa-3-isobutyryloxy- 4-methyl-1,6-cyclononanedione (Compound UK-2A-27L represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents benzyloxycarbonyl:

UK-2A (100 mg) was dissolved in 10 mL of methylene chloride. Pyridine (32 mg) and 83 mg of phosphorus pentachloride were added to the solution under ice cooling. The mixture was heated under reflux for 1.5 hr. Next, the reaction solution was cooked to -30℃. Methanol (10 mL), which had been previously cooled to 0℃, was added thereto, and a reaction was allowed to proceed at room temperature for 3 hr. Methylene chloride (50 mL) and 50 mL of saturated aqueous sodium hydrogencarbonate, which

had been previously cooled to 0℃, were added to the reaction solution, followed by separation. The aqueous layer was extracted twice with 20 mL of dichloromethane. The combined organic layers were dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was dissolved in 5 mL of methylene chloride. Pyridine (46 μl) and 84 μl of benzyloxycarbonyl chloride were added to the solution under ice cooling, and a reaction was allowed to proceed at room temperature for 20 min. The reaction solution was concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (hexane : ethyl acetate = 3 : 1) to obtain 45 mg (yield 48%) of the title compound.

[0052]

^{1}H-NMR ($CDCl_3$): δ=1.23 (6H, d, J=6.8, CH ($\underline{CH_3}$)$_2$), 1.29 (3H, d, J=6.2, 4-CH_3), 2.50~2.80 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.80~3.00 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.45 (1H, bs, H-8), 4.80~5.00 (2H, m, H-4, 7), 5.09 (2H, s, $C_6H_5\underline{CH_2}OCO$), 5.00~5.30 (2H, m, H-3, 8), 5.45 (1H, d, J=7.8, CONH), 7.09~7.33 (10H, m, $C_6H_5 \times 2$)

MS (EI): m/z=497 (M)

[0053]

<u>Example 3 (2R, 3R, 4S, 7S)-7-Amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione tosylate (Compound UK-2A-28L represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents a hydrogen atom):</u>

The title compound (yield 41%) was obtained in the same manner as in Example 1, except that isobutanol was used instead of methanol.

[0054]

<u>Example 4 (2R, 3R, 4S, 7S)-7-(2-Hydroxynicotinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-001 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 2-hydroxynicotinyl):</u>

UK-2A-29L (40 mg), 20 mg of 2-hydroxynicotinic acid, and 20 mg of 1-hydroxybenzotriazole were dissolved in 2 mL of pyridine. A solution of 29 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in tetrahydrofuran (THF, 2 mL) was added to the solution, and a reaction was allowed to proceed at room temperature for 3 hr. Dichloromethane and water were added to the reaction solution, followed by

separation. The organic layer was dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : hexane = 4 : 1) to obtain 28 mg (yield 78%) of the title compound.

[0055]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, d, J=7.0 CH ($\underline{CH_3}$)$_2$), 1.32 (3H, d, J=6.2, 4-CH_3), 2.58~2.73 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.89~3.05 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.63 (1H, bs, H-8), 4.94~5.00 (1H, m, H-4), 5.18~5.25 (2H, m, H-3, H-7), 5.40 (1H, bs, H-8), 6.55 (1H, t, J=6.8, H-5'), 7.12~7.29 (5H, m, C_6H_5), 7.63 (1H, dd, J=6.8, 2.2, H-4'), 8.57 (1H, dd, J=6.8, 2.2, H-6'), 10.31 (1H, d, CO<u>NH</u>, J=6.8), 12.78 (1H, s, OH)

MS (TSP): m/z=485 (M+H)

[0056]

<u>Example 5 (2R, 3R, 4S, 7S)-7-(3-Methoxysalicylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-002 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-methoxysalicyl):</u>

The title compound (yield 74%) was obtained in the same manner as in Example 4, except that 3-methoxysalicylic acid was used instead of 2-hydroxynicotinic acid.

[0057]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, d, J=7.3, CH ($\underline{CH_3}$)$_2$), 1.33 (3H, d, J=6.5, 4-CH_3), 2.60~2.73 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.92~3.05 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.63 (1H, bs, H-8), 3.90 (3H, s, OCH_3), 4.90~5.26 (3H, m, H-3, 4, 7), 5.18~5.25 (2H, m, H-3, H-7), 5.45 (1H, bs, H-8), 6.81~7.29 (8H, m, aromatic), 7.46 (1H, d, J=6.5, CO<u>NH</u>), 10.75 (1H, s, OH)

MS (TSP): m/z=514 (M+H)

[0058]

<u>Example 6 (2R, 3R, 4S, 7S)-7-(6-Hydroxypicolinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-005 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 6-hydroxypicolinyl):</u>

The title compound (yield 52%) was obtained in the same manner as in Example 4, except that 6-hydroxypicolinic acid was used instead of 2-hydroxynicotinic acid.

[0059]

^{1}H-NMR ($CDCl_3$): δ=1.05~1.34 (9H, m, CH ($\underline{CH_3}$)$_2$, 4-CH_3), 2.60~2.75 (2H,

m, CH $(CH_3)_2$, $CH_2C_6H_5$), 2.87~3.05 (2H, m, H-2, $CH_2C_6H_5$), 3.73 (1H, bs, H-8), 4.46 (1H, d, OH, J=8.9), 4.94~5.00 (1H, m, H-4), 5.18~5.32 (3H, m, H-3, 7, 8), 6.78 (1H, d, J=8.9, aromatic (pyridinering)), 7.12~7.30 (8H, m, aromatic (pyridinering, C_6H_5)), 7.58 (1H, dd, J=7.0, 2.2, aromatic (pyridinering)), 8.18 (1H, d, J=7.3 CONH)

MS (TSP): m/z=485 (M+H)

[0060]

Example 7 (2R, 3R, 4S, 7S)-7-(2,4-Dihydroxypyrimidine-5-carboxylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-006 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 2,4-dihydroxypyrimidine-5-carboxyl):

The title compound (yield 23%) was obtained in the same manner as in Example 4, except that 2,4-dihydroxypyrimidine-5-carboxylic acid was used instead of 2-hydroxynicotinic acid.

[0061]

^{1}H-NMR ($CDCl_3$): δ=1.05~1.32 (9H, m, 4-CH_3, CH $(CH_3)_2$), 2.59~2.72 (2H, m, CH $(CH_3)_2$, $CH_2C_6H_5$), 2.90~3.00 (2H, m, H-2, $CH_2C_6H_5$), 3.60 (1H, bs, H-8), 4.22 (1H, bd, OH), 4.90~5.40 (4H, m, H-3, 4, 7, 8), 7.11~7.26 (8H, m, C_6H_5), 8.51 (1H, s, aromatic (pyrimidinering)), 9.29 (1H, d, J=7.3, CONH)

MS (TSP): m/z=502 (M+H)

[0062]

Example 8 (2R, 3R, 4S, 7S)-7-(3-Hydroxy-2-methylquinoline-4-carboxylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-007 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-hydroxy-2-methylquinoline-4-carboxyl):

The title compound (yield 12%) was obtained in the same manner as in Example 4, except that 3-hydroxy-2-methyl-4-quinolinecarboxylic acid was used instead of 2-hydroxynicotinic acid.

[0063]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, 4-CH_3, CH $(CH_3)_2$), 2.77 (3H, s, CH_3 (quinoline)), 4.80~5.40 (4H, m, H-3, 4, 7, 8), 6.80~8.00 (10H, m, aromatic, CONH),

11.34 (1H, s, 3'-OH)

MS (TSP): m/z=549 (M+H)

[0064]

Example 9 (2R, 3R, 4S, 7S)-7-(2-Furoylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-009 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 2-furoyl):

The title compound (yield 50%) was obtained in the same manner as in Example 4, except that 2-pyromucic acid was used instead of 2-hydroxynicotinic acid.

[0065]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, dd, J=7.1, 1.1, CH ($\underline{CH_3}$)$_2$), 1.32 (3H, d, J=6.6, 4-CH_3), 2.57~2.73 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.88~3.04 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.57 (1H, bs, H-8), 4.96~5.02 (1H, m, H-4), 5.17~5.24 (2H, m, H-3, 7), 5.38 (1H, bs, H-8), 6.05 (1H, t, J=1.7, aromatic (furanring)), 7.02 (1H, d, J=7.7, CONH), 7.11~7.29 (6H, m, aromatic (benzenering, furanring)), 7.46 (1H, d, J=1.7, aromatic (furanring))

MS (TSP): m/z=458 (M+H)

[0066]

Example 10 (2R, 3R, 4S, 7S)-7-(3-Hydroxy-2-quinoxalinecarboxylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-010 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-hydroxy-2-methylquinoline-4-carboxyl):

The title compound (yield 27%) was obtained in the same manner as in Example 4, except that 3-hydroxy-2-quinoxalinecarboxylic acid was used instead of 2-hydroxynicotinic acid.

[0067]

^{1}H-NMR ($CDCl_3$): δ=1.23~1.37 (9H, m, J=7.1, 1.1, CH ($\underline{CH_3}$)$_2$, 4-CH_3), 2.60~2.75 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.90~3.10 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.66 (1H, bs, H-8), 4.99~5.51 (4H, m, H-3, 4, 7, 8), 7.13~8.12 (10H, m, CONH, aromatic (benzenering)), 11.78 (1H, s, -OH)

MS (TSP): m/z=536 (M+H)

[0068]

Example 11 (2R, 3R, 4S, 7S)-7-Salicylamino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-101 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents salicyl):

The title compound (yield 42%) was obtained in the same manner as in Example 4, except that salicylic acid was used instead of 2-hydroxynicotinic acid.

[0069]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.36 (9H, m, CH (CH_3)$_2$, 4-CH_3), 2.60~2.80 (2H, m, CH (CH_3)$_2$, $CH_2C_6H_5$), 2.91~3.00 (2H, m, $CH_2C_6H_5$, H-2), 3.60 (1H, bs, H-8), 4.98~5.27 (3H, m, H-3, 4, 7), 5.45 (1H, bs, H-8), 6.84~7.44 (10H, m, aromatic, CONH), 11.80 (1H, s, OH)

MS (TPS): m/z=484 (M+H)

[0070]

Example 12 (2R, 3R, 4S, 7S)-7-(3-Nitrosalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-102 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-nitrosalicyl):

The title compound (yield 66%) was obtained in the same manner as in Example 4, except that 3-nitrosalicylic acid was used instead of 2-hydroxynicotinic acid.

[0071]

^{1}H-NMR ($CDCl_3$): δ=1.23~1.37 (9H, m, CH (CH_3)$_2$, 4-CH_3), 2.60~2.80 (2H, m, CH (CH_3)$_2$, $CH_2C_6H_5$), 2.80~3.10 (2H, m, $CH_2C_6H_5$, H-2), 3.60 (1H, bs, H-8), 4.98 (1H, bs, H-4), 5.18~5.30 (2H, m, H-3, 7), 5.42 (1H, bs, H-8), 7.06~7.29 (6H, m, C_6H_5, H-6') , 8.27 (1H, d, J=7.6, H-5'), 8.45 (1H, d, J=7.6, H-4'), 8.76 (1H, bs, CONH)

MS (TPS): m/z=527 (M-H)

[0072]

Example 13 (2R, 3R, 4S, 7S)-7-(3-Aminosalicyl)amino-2-benzyl-5,9-dioxa-3- isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-103 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-aminosalicyl):

UK-2A-102 (50 mg) was dissolved in 25 mL of methanol. 10% palladium-carbon (5 mg) was added to the solution, followed by catalytic hydrogenation at room temperature

under normal pressure for one hr. The catalyst was removed by filtration. The filtrate was concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : hexane = 1 : 1) to give 16 mg (yield 34%) of the title compound.

[0073]

^{1}H-NMR ($CDCl_3$): δ=1.23 (6H, d, J=7.3, CH (<u>CH_3</u>)2), 1.33 (3H, d, J=5.9, 4-CH_3), 2.60~2.80 (2H, m, <u>CH</u> $(CH_3)_2$, <u>CH_2</u>C_6H_5), 2.92~3.00 (2H, m, <u>CH_2</u>C_6H_5, H-2), 3.60 (1H, bs, H-8), 4.00 (2H, bs, NH_2), 4.98 (1H, bs, H-4), 5.00~5.50 (2H, m, H-3, 4, 7, 8), 5.42 (1H, bs, H-8), 6.66~7.29 (9H, m, aromatic, CONH), 12.00 (1H, s, OH)

MS (TSP): m/z=499 (M+H)

[0074]

<u>Example 14 (2R, 3R, 4S, 7S)-7-(3-Formylaminosalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-104 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-formylaminosalicyl):</u>

UK-2A-103 (8.8 mg) was dissolved in 1 mL of methylene chloride. Formic acid (0.5 mL) and 0.1 mL of acetic anhydride were added sequentially, and a reaction was followed to proceed at room temperature for 30 min. Methylene chloride and water were added thereto, followed by separation. The organic layer was dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : hexane = 1 : 1) to give 4.2 mg (yield 44%) of the title compound.

[0075]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, m, CH (<u>CH_3</u>)$_2$, 4-CH_3), 2.60~2.80 (2H, m, <u>CH</u> $(CH_3)_2$, <u>CH_2</u>C_6H_5), 2.80~3.10 (2H, m, <u>CH_2</u>C_6H_5, H-2), 3.59 (1H, bs, H-8), 5.00~5.26 (4H, m, H-3, 4, 7, 8), 6.66~7.29 (8H, m, aromatic), 12.00 (1H, s, OH)

MS (TSP): m/z=527 (M+H)

[0076]

<u>Example 15 (2R, 3R, 4S, 7S)-7-(5-Nitrosalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-105 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 5-nitrosalicyl):</u>

The title compound (yield 84%) was obtained in the same manner as in Example 4, except that 5-nitrosalicylic acid was used instead of 2-hydroxynicotinic acid.

[0077]

^{1}H-NMR ($CDCl_3$): δ=1.22~1.43 (9H, m, CH ($\underline{CH_3}$)$_2$, 4-CH_3), 2.61~2.75 (2H, m, $\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 2.90~3.01 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.68 (1H, bs, H-8), 4.90~5.40 (4H, m, H-3, 4, 7, 8), 7.00~7.30 (6H, m, H-3'), 7.58 (1H, d, J=6.5, CONH), 8.27 (1H, dd, J=8.9, 2.2, H-4'), 8.46 (1H, d, J=2.2, H-6')

MS (TSP): m/z=527 (M-H)

[0078]

Example 16 (2R, 3R, 4S, 7S)-7-(5-Aminosalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-106 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 5-aminosalicyl):

The title compound (yield 49%) was obtained in the same manner as in Example 12, except that UK-2A-105 was used instead of UK-2A-102.

[0079]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, m, CH ($\underline{CH_3}$)$_2$, 4-CH_3), 2.58~2.80 (2H, m, $\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 2.88~3.04 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.58 (1H, bs, H-8), 4.90~5.40 (4H, m, H-3, 4, 7, 8), 6.70~7.30 (9H, m, aromatic, CONH)

MS (TSP): m/z=499 (M+H)

[0080]

Example 17 (2R, 3R, 4S, 7S)-7-(4-Chlorosalicyl)amino-2-benzyl-5,9-dioxa-3- isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-109 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 4-chlorosalicyl):

The title compound (yield 26%) was obtained in the same manner as in Example 4, except that 4-chlorosalicylic acid was used instead of 2-hydroxynicotinic acid.

[0081]

^{1}H-NMR ($CDCl_3$): δ=1.23 (6H, d, J=7.0, CH ($\underline{CH_3}$)$_2$), 1.34 (3H, d, J=6.5, 4-CH_3), 2.40~3.00 (4H, m, $\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$, H-2), 3.60 (1H, bs, H-8), 4.90~5.60 (4H, m, H-3, 4, 7, 8), 6.83~7.36 (9H, m, aromatic, CONH), 11.99 (1H, s, OH)

MS (TSP): m/z=518 (M+H)

[0082]

Example 18 (2R, 3R, 4S, 7S)-7-(5-Chlorosalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-110 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 5-chlorosalicyl):

The title compound (yield 60%) was obtained in the same manner as in Example 4, except that 5-chlorosalicylic acid was used instead of 2-hydroxynicotinic acid.

[0083]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, m, CH $(\underline{CH_3})_2$, 4-$\underline{CH_3}$), 2.50~3.00 (4H, m, $\underline{CH}$, $(CH_3)_2$, $\underline{CH_2}C_6H_5$, H-2), 3.60 (1H, bs, H-8), 4.98~5.42 (4H, m, H-3, 4, 7, 8), 6.90~8.01 (9H, m, aromatic, CONH), 11.71 (1H, s, OH)

MS (TSP): m/z=518 (M+H)

[0084]

Example 19 (2R, 3R, 4S, 7S)-7-(4-Methoxysalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-112 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 4-methoxysalicyl):

The title compound (yield 37%) was obtained in the same manner as in Example 4, except that 4-methoxysalicylic acid was used instead of 2-hydroxynicotinic acid.

[0085]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, m, CH $(\underline{CH_3})_2$, 4-CH_3), 2.60~2.80 (2H, m, $\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 2.80~3.10 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.60 (1H, bs, H-8), 3.80 (3H, s, OMe), 4.90~5.50 (4H, m, H-3, 4, 7, 8), 6.50~7.40 (8H, m, aromatic), 12.10 (1H, s, OH)

TSP-MS: m/z=514 (M+H)

[0086]

Example 20 (2R, 3R, 4S, 7S)-7-(3,5-Dinitrosalicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-114 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3,5-dinitrosalicyl):

The title compound (yield 98%) was obtained in the same manner as in Example 4, except that 3,5-dinitrosalicylic acid was used instead of 2-hydroxynicotinic acid.

[0087]

^{1}H-NMR ($CDCl_3$): δ=1.00~1.30 (9H, m, CH $(\underline{CH_3})_2$, 4-CH_3), 2.50~2.70 (2H, m,

$\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 2.70~2.90 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.60 (1H, bs, H-8), 4.60~5.20 (4H, m, H-3, 4, 7, 8), 7.00~7.30 (5H, m, $CH_2\underline{C_6H_5}$), 7.60 (1H, bs, CONH), 8.60~8.90 (2H, m, aromatic (3, 5-Dinitrosalicyl)

MS (TSP): m/z=573 (M+H)

[0088]

Example 21 (2R, 3R, 4S, 7S)-7-(3-(N,N-Dimethylamino)salicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-115 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-(N,N-dimethylamino) salicyl):

UK-2A-102 (30 mg) was dissolved in 5 mL of methanol. 40% formalin (1 mL) and 3 mg of 10% palladium-carbon were added to the solution, followed by hydrogenation at room temperature under normal pressure for 8 hr. The catalyst was then removed by filtration. The filtrate was concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (methylene chloride : ethyl acetate = 3 : 1) to give 8.0 mg (yield 27%) of the title compound.

[0089]

^{1}H-NMR ($CDCl_3$): δ=1.29~1.34 (9H, m, CH $(\underline{CH_3})_2$, 4-CH_3), 2.60~2.73 (2H, m, $\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 2.73 (6H, s, N $(CH_3)_2$), 2.92~3.00 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.60 (1H, bs, H-8), 4.90~5.50 (4H, m, H-3, 4, 7, 8), 6.88 (1H, t, J=7.6, H-4'), 7.11~7.29 (6H, m, C_6H_5, H-5'), 7.51 (1H, d, J=9.5, H-6'), 7.96 (1H, d, J=8.2, CONH)

MS (TSP): m/z=527 (M+H)

[0090]

Example 22 (2R, 3R, 4S, 7S)-7-(5-(N,N-Dimethylamino)salicyl)amino-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-116 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 5-(N,N-dimethylamino)salicyl):

The title compound (yield 26%) was obtained in the same manner as in Example 21, except that UK-2A-105 was used instead of UK-2A-102.

[0091]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, m, CH $(\underline{CH_3})_2$, 4-CH_3), 2.50~2.80 (2H, m,

$\underline{CH}$ $(CH_3)_2$, $\underline{CH_2}C_6H_5$), 2.87 (6H, s, N $(CH_3)_2$), 2.80~3.00 (2H, m, $\underline{CH_2}C_6H_5$, H-2), 3.61 (1H, bs, H-8), 4.90~5.50 (4H, m, H-3, 4, 7, 8), 6.67~7.30 (9H, m, aromatic, CONH), 11.04 (1H, s, OH)

MS (TSP): m/z=527 (M+H)

[0092]

Example 23 (2R, 3R, 4S, 7S)-7-(3-Acetoxy-4-methoxypicolinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound Acetyl-UK-2A represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-acetoxy-4-methoxypicolinyl):

UK-2A (6.32 g) was dissolved in 80 mL of pyridine. Acetate anhydride (2.5 mL) was added to the solution under ice cooling, and a reaction was allowed to proceed at room temperature for 3 hr. The reaction solution was concentrated and dried under the reduced pressure to give 6.7 g (yield 100%) of the title compound as a white solid.

[0093]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, d, J=6.9, CH $(\underline{CH_3})_2$), 1.30 (3H, d, J=6.2, 4-CH_3), 2.38 (3H, s, $OCOCH_3$), 2.61 (1H, septet, J=6.9, $\underline{CH}$ $(CH_3)_2$), 2.70 (1H, d, J=11.4, $\underline{CH_2}C_6H_5$), 2.87~2.99 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.57 (1H, bs, H-8), 3.90 (3H, s, OCH_3), 4.96 (1H, dp, J=9.5, 6.2, H-4), 5.14 (1H, t, J=8.4, H-7), 5.20 (1H, t, J=9.5, H-3), 5.34 (1H, bs, H-8), 7.01 (1H, d, J=5.5, H-5'), 7.11~7.28 (5H, m, C_6H_5), 8.32 (1H, d, J=5.5, H-6'), 8.63 (1H, d, CONH, J=8.4)

MS (TSP): m/z=557 (M+H)

[0094]

Example 24 (2R, 3R, 4S, 7S)-7-(3-Benzoyloxy-4-methoxypicolinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-204 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-benzoyloxy-4-methoxypicolinyl):

UK-2A (50 mg) was dissolved in 5 mL of pyridine. Benzoyl chloride (27 mg) was added to the solution under ice cooling, and a reaction was allowed to proceed at room temperature for 2 hr. The reaction solution was diluted with methylene chloride.

The diluted solution was washed with water twice, dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : hexane = 3 : 1) to give 33 mg (yield 55%) of the title compound.

[0095]

^{1}H-NMR ($CDCl_3$): δ=1.22 (6H, d, J=7.1, CH ($\underline{CH_3}$)$_2$), 1.27 (3H, d, J=6.0, 4-CH_3), 2.50~2.70 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.80~3.00 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.60 (1H, bs, H-8), 3.89 (3H, s, Ome), 4.90~5.30 (4H, m, H-3, 4, 7, 8), 7.06 (1H, d, J=5.5, H-5'), 7.09~7.26 (5H, m, $CH_2C_6\underline{H_5}$), 7.48~7.66, 8.20~8.23 (3H, 2H, m, $COC_6\underline{H_5}$), 8.38 (1H, d, J=5.5, H-6'), 8.66 (1H, d, J=8.2, CONH)

MS (TSP): m/z=619 (M+H)

[0096]

Example 25 (2R, 3R, 4S, 7S)-7-(3-Isopropyloxycarbonyloxy-4-methoxypicolinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-207 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-isopropyloxycarbonyloxy-4-methoxypicolinyl):

UK-2A (50 mg) was dissolved in 5 mL of methylene chloride. Triethylamine (1 mL) and 1 mL of isopropyl chloroformate were added to the solution under ice cooling, and a reaction was allowed to proceed at room temperature for 1 hr. The reaction solution was diluted with methylene chloride. The diluted solution was washed with water twice, dried over magnesium sulfate, and then concentrated under the reduced pressure to give 58 mg (yield 100%) of the title compound.

[0097]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (15H, m, OCOCH ($\underline{CH_3}$)$_2$, OCH ($\underline{CH_3}$)$_2$, 4-CH_3), 2.50~2.80 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_5$), 2.80~3.10 (2H, m, H-2, $\underline{CH_2}C_6H_5$), 3.60 (1H, bs, H-8), 3.92 (3H, s, OMe), 4.93~5.40 (5H, m, O$\underline{CH}$ (CH_3)$_2$, H-3, 4, 7, 8), 7.02 (1H, d, J=5.5, H-5'), 7.11~7.29 (5H, m, C_6H_5), 8.33 (1H, d, J=5.5, H-6'), 8.58 (1H, d, J=8.2, CONH)

MS (TSP): m/z=601 (M+H)

[0098]

Example 26 (2R, 3R, 4S, 7S)-7-(3-(3-Methoxycarbonylpropionyloxy)-4-methoxypicolinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-208 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-(3-methoxycarbonylpropionyloxy)-4-methoxypicolinyl):

A methylene chloride (20 mL) solution of UK-2A (100 mg) and triethylamine (0.27 mL) was dropped to a mixture of succinic acid chloride (0.22 mL) and methylene chloride (5 mL) under ice cooling, and a reaction was allowed to proceed at room temperature for 2 hr. The reaction solution was ice cooled for the second time, and a reaction was allowed to proceed at room temperature for 1 hr. The reaction solution was diluted with methylene chloride. The diluted solution was washed with water twice, dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : hexane = 1 : 1) to give 53 mg (yield 44%) of the title compound.

[0099]

^{1}H-NMR ($CDCl_3$): δ=1.23 (6H, d, J=7.1, CH $(\underline{CH_3})_2$), 1.31 (3H, d, J=6.0, 4-CH_3), 2.50~3.10 (8H, m, $\underline{CH}$ $(CH_3)_2$, CO$\underline{CH_2CH_2}$CO, $\underline{CH_2}C_6H_5$, H-2), 3.72 (3H, s, $COOCH_3$), 3.90 (3H, s, Ome), 4.90~5.40 (4H, m, H-3, 4, 7, 8), 7.00 (1H, d, J=5.4, H-5'), 7.11~7.28 (5H, m, C_6H_5), 8.32 (1H, d, J=5.4, H-6'), 8.62 (1H, d, J=8.4, CONH)

MS (FAB): m/z=629 (M+H)

[0100]

Example 27 (2R, 3R, 4S, 7S)-7-(3-(3-Benzyloxycarbonylpropionyloxy)-4-methoxypicolinylamino)-2-benzyl-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-209 represented by formula (1), wherein R_1 represents isobutyryl; and R_2 represents 3-(3-benzyloxycarbonylpropionyloxy)-4-methoxypicolinyl):

UK-2A (100 mg), 49 mg of monobenzylester succinate and 55 mg of 4-dimethylamino pyridine were dissolved in 20 mL of methylene chloride. Dicyclohexylcarbodiimido (60 mg) was added to the solution under ice cooling, and a reaction was allowed to proceed at room temperature for 6 hr. The reaction solution was subjected to filtration. The filtrate was washed with 1N hydrochloric acid,

saturated aqueous sodium hydrogencarbonate, and water in that order, dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : hexane = 1 : 1) to give 92 mg (yield 69%) of the title compound.

[0101]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, d, J=7.1, CH (<u>CH_3</u>)$_2$,) 1.30 (3H, d, J=6.0, 4-CH_3), 2.58~3.07 (8H, m, <u>CH</u> $(CH_3)_2$, CO<u>CH_2CH_2</u>CO, <u>CH_2</u>C_6H_5, H-2), 3.55 (1H, bs, H-8), 3.86 (3H, s, OMe), 5.16 (2H, s, COO<u>CH_2</u>C_6H_5), 4.90~5.40 (4H, m, H-3, 4, 7, 8), 6.99 (1H, d, J=5.4, H-5'), 7.11~7.37 (10H, m, C_6H_5×2), 8.31 (1H, d, J=5.4, H-6'), 8.61 (1H, d, J=8.4, CONH)

MS (FAB): m/z=705 (M+H)

[0102]

<u>Example 28 (2R, 3R, 4S, 7S)-7-(3-Hydroxy-4-methoxypicolinyl)amino-2-(4-nitrobenzyl)-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-401 represented by formula (2), wherein R_1 represents isobutyryl; and R_2 represents nitro:</u>

UK-2A (30 mg) was dissolved in 1.5 mL of methylene chloride. The solution was cooled to −20℃. Fuming nitric acid (specific gravity 1.52) (0.3 mL) was added to the solution, and a reaction was allowed to proceed at room temperature for 2 hr. The reaction solution was diluted with cooled methylene chloride. The diluted solution was washed with saturated aqueous sodium hydrogencarbonate and water in that order, dried over magnesium sulfate, and then concentrated under the reduced pressure to give 32 mg (yield 98%) of the title compound.

[0103]

^{1}H-NMR ($CDCl_3$): δ=1.26 (6H, d, J=7.1, CH, (<u>CH_3</u>)$_2$), 1.34 (3H, d, J=6.0, 4-CH_3), 2.63~2.90 (2H, m, <u>CH</u> $(CH_3)_2$, <u>CH_2</u>$C_6H_4NO_2$), 2.96~3.12 (2H, m, <u>CH_2</u>$C_6H_4NO_2$, H-2), 3.65 (1H, bs, H-8), 3.94 (3H, s, OCH_3), 4.97~5.03 (1H, m, H-4), 5.19~5.30 (3H, m, H-3, 7, 8), 6.88 (1H, d, J=4.9, H-5'), 7.31 (2H, d, J=8.3, $C_6H_4NO_2$), 7.98 (1H, d, J=4.9, H-6') 8.13 (2H, d, J=8.3, $C_6H_4NO_2$), 8.60 (1H, d, J=8.2, CONH), 11.73 (1H, s, OH)

MS (TSP): m/z=560 (M+H)

[0104]

Example 29 (2R, 3R, 4S, 7S)-7-(3-Hydroxy-4-methoxypicolinyl)amino-2-(4-aminobenzyl)-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-402 represented by formula (2), wherein R_1 represents isobutyryl; and R_2 represents amino:

UK-2A-401 (220 mg) was dissolved in 50 mL of ethanol. 10% palladium-carbon (22 mg) was added to the solution, followed by hydrogenation at room temperature under normal pressure for 6 hr. The catalyst was removed by filtration. The filtrate was then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (chloroform : methanol = 20 : 1) to give 151 mg (yield 72%) of the title compound.

[0105]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, d, J=7.1, CH ($\underline{CH_3}$)$_2$), 1.34 (3H, d, J=6.0, 4-CH_3), 2.50~2.70 (2H, m, $\underline{CH}$ (CH_3)$_2$, $\underline{CH_2}C_6H_4NH_2$), 2.80~3.00 (2H, m, $\underline{CH_2}C_6H_4NH_2$, H-2), 3.61 (1H, bs, H-8), 3.94 (3H, s, OCH_3), 4.90~5.10 (1H, m, H-4), 5.10~5.40 (3H, m, H-3, 7, 8), 6.58 (2H, d, J=8.2, $C_6\underline{H_4}NH_2$), 6.87 (1H, d, J=5.5, H-5'), 6.91 (2H, d, J=8.2, $C_6\underline{H_4}NH_2$), 7.99 (1H, d, J=5.5, H-6'), 8.59 (1H, d, J=8.2, CONH), 11.79 (1H, s, OH)

MS (TSP): m/z=530 (M+Z)

[0106]

Example 30 (2R, 3R, 4S, 7S)-7-(3-Hydroxy-4-methoxypicolinyl)amino-2-(4-formylaminobenzyl)-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-403 represented by formula (2), wherein R_1 represents isobutyryl; and R_2 represents formylamino:

UK-2A-402 (29 mg) was dissolved in 1 mL of methylene chloride. Formic acid (0.5 mL) and 0.1 mL of acetic anhydride were added sequentially to the solution, and a reaction was allowed to proceed at room temperature for 30 min. The reaction solution was diluted with methylene chloride. The diluted solution was washed with water, dried over magnesium sulfate, and then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (chloroform : methanol = 10 : 1) to give 14 mg (yield 46%) of the title compound.

[0107]

^{1}H-NMR ($CDCl_3$): δ=1.20~1.40 (9H, m, CH (<u>CH_3</u>)$_2$, 4-CH_3), 2.60~2.80 (2H, m, <u>CH</u> (CH_3)$_2$, <u>CH_2</u>C_6H_4NHCHO), 2.80~3.00 (2H, m, <u>CH_2</u>C_6H_4NHCHO, H-2), 3.60 (1H, bs, H-8), 3.94 (3H, s, OCH_3), 4.90~5.40 (1H, m, H-3, 4, 7, 8), 6.88 (1H, d, J=5.1, H-5'), 6.97~8.64 (4H, m, C_6<u>H_4</u>NHCHO), 7.99 (1H, d, J=5.1, H-6'), 11.79 (1H, s, OH)

MS (TSP): m/z=558 (M+Z)

[0108]

<u>Example 31 (2R, 3R, 4S, 7S)-7-(3-Hydroxy-4-methoxypicolinyl)amino-2-(4-(N,N-dimethylamino)benzyl)-5,9-dioxa-3-isobutyryloxy-4-methyl-1,6-cyclononanedione (Compound UK-2A-404 represented by formula (2), wherein R_1 represents isobutyryl; and R_2 represents N,N-dimethylamino:</u>

UK-2A-402 (30 mg) was dissolved in 5 mL of ethanol. 40% formalin (1 mL) and 3 mg of 10% palladium-carbon were added to the solution, followed by hydrogenation at room temperature under normal pressure for 4 hr. The catalyst was removed by filtration. The filtrate was then concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (chloroform : methanol = 40 : 1) to give 21 mg (yield 66%) of the title compound.

[0109]

^{1}H-NMR ($CDCl_3$): δ=1.24 (6H, d, J=7.1, CH (<u>CH_3</u>)$_2$, 1.32 (3H, d, J=6.0, 4-CH_3), 2.50~2.70 (2H, m, <u>CH</u> (CH_3)$_2$, <u>CH_2</u>C_6H_4N (CH_3)$_2$), 2.80~3.00 (2H, m, <u>CH_2</u>C_6H_4N (CH_3)$_2$, H-2), 2.90 (6H, s, N (CH_3)$_2$), 3.60 (1H, bs, H-8, 3.94 (3H, s, OCH_3), 4.90~5.40 (1H, m, H-3, 4, 7, 8), 6.64 (2H, d, J=8.8, CH_2C_6<u>H_4</u>N (CH_3)$_2$), 6.87 (1H, d, J=5.1, H-5'), 6.99 (2H, d, J=8.8, CH_2C_6<u>H_4</u>N (CH_3)$_2$), 7.99 (1H, d, J=5.1, H-6'), 8.50 (1H, d, J=8.2, CONH), 11.80 (1H, s, OH)

MS (TSP): m/z=558 (M+H)

[0110]

<u>Test Example 1 Evaluation test on antifungal activity</u>

The antifungal activity was tested using <u>Saccharomyces cerevisiae IFO 0203</u> by the following method.

[0111]

(1) Medium

Sabouraud medium (pH 5.5-6.0)

Glucose	40g/L
Polypeptone	10g/L

Assay medium (pH unadjusted)

Yeastext. (DIFCO)	10g/L
Polypeptone	20g/L
Glycerol	30g/L
Bacto-agar (DIFCO)	20g/L

[0112]

(2) Preparation of assay fungi

One platinum loop of the fungi was inoculated into the Sabouraud liquid medium (10 mL/sextant testing tube), followed by shaking cultivation at 26℃ for 24 hr (360 rpm; tube shaker).

[0113]

(3) Preparation of assay plate

A lower layer (agar 20g/L) was spread on an assay plate. The assay medium for an upper layer was heat melted, and then cooled to 45 to 50℃. The assay fungi (3 to 4 mL) was inoculated into 150 mL assay medium/250 mL Erlenmeyer flask. After solidification of the lower layer was confirmed, the upper layer medium was spread thereon.

[0114]

(4) Evaluation of samples

The evaluation samples were penetrated into a sterilized paper disk and put on the assay plate, followed by cultivation at 26℃ for one to two days to measure the inhibition zone diameter. The results are summarized in Table 1.

[0115]

[Table 1]

Table 1 Results of evaluation test on antifungal activity (measured value of inhibition

zone diameter in mm)

Compound	Amount of sample used, (μg)			
	0.025	0.05	0.125	0.25
Antimycin A	11.5	13.5	16	18
UK-2A	19	21.5	22.5	26
UK-2A-001	0	12	16	17
UK-2A-010	14	17.5	20	23.5
UK-2A-101	8	8	11	12
UK-2A-104	8	11.5	16	16.5
UK-2A-115	8	8	12	13.5
UK-2A-207	0	9	13	16
UK-2A-208	15	18.5	22	24.5
UK-2A-200	10	12	13.5	17.5
UK-2A-401	12	14.5	18	20
UK-2A-404	0	11	14.5	18.5

[0116]

Test Example 2 Test on plant disease protective effect (test on effect of protecting cucumber against downy mildew)

Cucumber seedlings (variety: Suyo) of first leaf development stage raised in each of plastic pots containing compost were provided. A predetermined amount of test compound was dissolved in acetone. Tween 20 and water were added to the solution to prepare an agent containing 10% of acetone and 0.05% of tween 20.

This agent was applied in an amount of 5 mL per three pots by means of a spray gun. The agent was air dried. Thereafter, a conidial suspension, which had been previously prepared by scraping lesion portions on the undersurface of cucumber suffering from cucumber downy mildew (pathogenic fungi: Peseudoperonospora cubensis), was evenly inoculated by spraying. The pots were then kept under moist chamber conditions at 20℃ for 24 hr to perform infection. Thereafter, they were transferred to an environment control room kept at 18℃ at night and 22℃ in the

daytime to induce the disease. Seven days after the inoculation, disease on the blade of the leaf was evaluated based on a disease index in terms of the percentage lesion area [0 (not diseased) to 5 (not less than 75% of the leaf area diseased)], and the incidence of disease and the protective value were calculated by the following equations. The results were summarized in Table 2.

[0117]

Incidence of disease = Σ (number of disease for each severity $\times$ index)/(5 $\times$ number of investigated leaves) $\times$ 100

Protective value = (1 - incidence of disease in treated plot/number of lesions in nontreated plot) $\times$ 100

[0118]

[Table 2]

Table 2 Test results on effect of protecting cucumber against downy mildew

Compound	Concentration (ppm)	Protective value
Not applied	-	0
UK-2A	200	13
UK-2A-001	200	78
UK-2A-005	200	100
UK-2A-102	200	100
UK-2A-103	200	88
UK-2A-107	200	100

[0119]

The novel compounds according to the present invention do not have any phytotoxicity, even when they were applied at a concentration of 200 ppm, and had higher protective values than UK-2A.

[0120]

Test Example 3 Test on plant disease protective effect (confirmation test on persistence of the effect of protecting cucumber against anthracnose)

Cucumber seedlings (variety: Suyo) of first leaf development stage raised in

each of plastic pots containing compost were provided. An agent prepared in the same manner as in Test Example 2 was applied in an amount of 5 mL per three pots by means of a spray gun. Thereafter, test were conducted under the following conditions.

[0121]

Experimental plot 1

After the air drying, the pots were placed in an environment control room under an indoor fluorescent lamp for one day. On the following day, a conidial suspension of cucumber anthracnose fungi (Colletotricum lagenarium), which had been previously cultured in a potato soup agar medium, was evenly inoculated by spraying. The pots were then kept under moist chamber conditions at 26℃ for 24 hr to perform infection. Thereafter, they were transferred to a glass greenhouse kept at 18℃ at night and 25℃ in the daytime. Seven days after the inoculation, disease on the blade of the leaf was evaluated.

[0122]

Experimental plot 2

After the air drying, the pots were placed outdoors under sunlight in the daytime (8 hr) or in a glass greenhouse. On the following day, a conidial suspension of cucumber anthracnose fungi (Colletotricum lagenarium), which had been previously cultured in a potato soup agar medium, was evenly inoculated by spraying. The pots were then kept under moist chamber conditions at 26℃ for 24 hr to perform infection. Thereafter, they were transferred to a glass greenhouse kept at 18℃ at night and 25℃ in the daytime. Seven days after the inoculation, disease on the blade of the leaf was evaluated.

[0123]

The incidence of disease and the protective value in each experimental plot were calculated by the same method as in Test Example 2. The results were summarized in Table 3.

[0124]

[Table 3]

Table 3 Test results on persistence of the effect of protecting cucumber against

anthracnose

Compound	Concentration (ppm)	Protective value	
		Plot 1	Plot 2
UK-2A	10	93	27
	30	100	60
Acetyl-UK-2A	10	93	60
	30	93	80

[0125]

Test Example 3 shows that Acetyl-UK-2A had much higher protective values than UK-2A and that, for the residual effect under sunlight which is most important for practical use, Acetyl-UK-2A was apparently much superior to UK-2A.

[0126]

Test Example 4 Photostability test (percentage residue as determined by HPLC)

In consideration of use in agricultural chemicals, the following test was carried out to obtain data on photostability against exposure to sunlight.

[0127]

Date and time of test:

First: 5 hr from 12:00 to 17:00 on May 26, 1997

Second: 6 hr from 10:00 to 16:00 on May 28, 1997

Place: For both tests, Odawara-shi, Kanagawa

Weather: For both tests, fine

[0128]

Preparation of samples: UK-2A (25 mg) and Acetyl-UK-2A (25 mg) each were dissolved in 5 mL of acetone and then spread on a laboratory dish having a diameter of about 9 cm. Acetone was shortly evaporated. As a result, each sample was brought into a white thin film. The films thus obtained were exposed to sunlight.

After the completion of exposure to sunlight, the residual amount of UK-2A and the residual amount of Acetyl-UK-2A were quantitatively determined by HPLC. The results were as summarized in Table 4.

[0129]

[Table 4]

Table 4 Residual amount (%) of UK-2A and Acetyl-UK-2A after exposure to sunlight

	UK-2A	Acetyl-UK-2A
First test	33	98
Second test	64	93

[0130]

For UK-2A, it was demonstrated that O-acetylation of the hydroxyl group at the 3'-position markedly improved the photostability. This fact supports the results of the test on persistence of the effect of protecting cucumber against anthracnose in Test Example 3.

[0131]

Test Example 5 Photostabiilty test (effect of protecting rice seedlings against blast)

Rice seedlings of three-leaf stage (variety: Koshihikari) raised outdoors in an upland field bed (1 m × 1 m) for rice seedlings were covered with a vinyl tunnel, only at night, with ears of rice suffering from blast being suspended (height 40 cm) to infect rice seedlings with blast. After the incipient infection was confirmed, agent solutions having an agent concentration of 200 ppm were applied in an amount of 100 mL per m^2 by means of a sprayer. For one week after the application of the agent, the rice seedlings were covered with the vinyl tunnel at night to promote the infection. 19 days after the application of the agent, the lesion area of the leaf was measured, and the protective value was calculated by the following equation. The results were as summarized in Table 5.

[0132]

Protective value = (1 - average lesion area of treated plot)/lesion area of untreated plot × 100

[0133]

[Table 5]

Table 5 Test results on protection of rice seedlings againat blast

Compound	Concentration (ppm)	Protective valus
Not applied	-	0
UK-2A	200	63
Acetyl-UK-2A	200	95

[0134]

There was a substantial correlation between the results obtained in this test example and the residual amount after exposure to sunlight in Test Example 4. Specifically, the test example demonstrated that, also on the protection of rice seedlings against blast, for UK-2A, O-acetylation of the hydroxyl group at the 3'-position markedly improved the photostability.

[0135]

[The Effect of the Invention]

Compounds obtained in the present invention had high antifungal activity and safety and could be used to produce antifungal agents comprising the compounds. Moreover, the present inventors could succeed in finding an effective method for producing the compounds, and the preparation of such antifungal agents were made easier.

Furthermore, compounds obtained in the present invention demonstrated improved effects as antifungal agents for agricultural and gardening applications and performed high effects in the field.

ABSTRACT

[Summary]

[Object]

Disclosed are novel carboxylic acid amido compounds having antifungal activity, a method for producing the same, and a use of the same.

[Method Of Solution]

By using UK-2, a fermented product, as a lead compound and by using chemical synthetic methods, the present inventors succeeded in finding novel derivatives having aromatic carboxylic acid and a nine-membered ring dilactone that has an amino group. The present inventors evaluated antifungal activity of the novel derivatives, and their usefulness became apparent.